Coal Tar Fungicides for the Control of Mouldy Rot

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COAL TAR FUNGICIDES FOR THE CONTROL OF MOULDY ROT

An official scheme for proprietary fungicides intended for use against mouldy rot (Ceratostomella fimbriata) has been in operation since 1931, the testing work involved being carried out by the Pathological Division of the Rubber Research Institute of Malaya and the first paper in this Communication, by Mr F.W. Hutchison, describes the testing methods at present employed.

After the war it became apparent that there were considerable differences in effectiveness between approved products, making a stricter scheme of approving fungicides desirable and if possible drawing up a specification. The first step was the fractionation of the crude tar oil preparations and their isolation and identification but this was outside the scope of the Pathological Division. Through the Agricultural Research Council of Great Britain the Board of the Institute arranged to have the work done at the University of Bristol, Agricultural and Horticultural Research Station, Long Ashton (Director: Professor T.W. Wallace, C.B.E., M.C., D.Sc., V.M.H., F.R.S.). Mr G.V. Coles was engaged to carry out the chemical investigations while biological tests were done by Dr R.J.W. Byrde, a member of the staff of Long Ashton Research Station. The work was under the general supervision of Dr J.T. Martin, Head of the Department of Chemistry of Insecticides and Fungicides. The papers included in this Communication, excepting that on the 'Testing of Coal Tar Fungicides for the Control of Mouldy Rot', represent the results of investigations carried out under the scheme between 1952 and 1954. The two papers on 'the Fungicidal Properties of Coal Tar Distillates' are reprinted from the Annual Reports of the Long Ashton Research Station for 1953 and 1954 where they appeared as Progress Reports. The paper on 'A Method of Analysis of Coal Tar Fungicides' is reprinted from the Journal of the Science of Food and Agriculture Volume 7, Number 1, 1956.

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I TESTING OF COAL TAR FUNGICIDES FOR THE CONTROL OF MOULDY ROT

(*Ceratostomella fimbriata*)

By

F.W. HUTCHISON

The establishment of an approval scheme in 1931 for fungicides for the treatment of mouldy rot C.fimbriata, led to the setting up of a method of testing such materials and assessing their efficacy.

The methods at present in use for tar oil fungicides are described and the need for a specification discussed.

Mouldy Rot (*Ceratostomella fimbriata*), a disease which attacks the tapping panel of rubber trees, was first reported in Malaya in 1916 and has ever since continued to be troublesome.

Under the Agricultural Pests Enactments of 1926 and 1935 mouldy rot was gazetted as a notifiable disease and legal enforcement of its treatment meant legal recognition of what constituted effective treatment. The unsatisfactory position in regard to effective fungicides led to the formulation of a scheme for testing and approving submitted materials. The scheme was originally formulated by A. Sharples of the Rubber Research Institute and in 1931 it was approved by the Federated Malay States Government and gazetted. The Gazette notification intimating the introduction of the scheme is reproduced in the *Appendix* on page 458.

A history of the work leading up to the establishment of the scheme was prepared by BEELEY (1936), who also described the form of the tests. A later paper (HUTCHISON 1953) dealt with the scheme in its post-war form and wider aspect.

Following the end of the war in Malaya in 1945, rehabilitation of estates entailed extensive treatment of mouldy rot but the shortage of suitable fungicides, seriously hampered this work. By 1947 however, most of the fungicides which had been approved previously were again on the local market but a number of complaints were received alleging that several were ineffective.

The scheme for approval of fungicides made no provision for a declaration of composition (since 1952 this has been required), all that it required was a guarantee from the manufacturer that the composition of his preparation would remain unchanged from that submitted for test. When complaints of ineffectiveness of preparations were reported the unsatisfactory nature of this arrangement became apparent and field re-tests of all the approved materials were carried out.

At this time all fungicides on the approved list were tar oils and to make the test comparable all were tested at a concentration of five per cent in water. Wide differences in activity were recorded (ALTSON 1950).

As the result of this disclosure consideration was given to revising the regulations by insisting on a declaration of composition and compliance with a specification. Through lack of information of the factors which impart fungicidal activity to coal tar fungicides however, it was clear that no specification could be put up at this stage.

APPROVAL SCHEME

Although mouldy rot is no longer a notifiable disease under the Agricultural Pests and Noxious Plants Ordinance 1953, due to its universal distribution, the scheme for approval of fungicides for its treatment is continued.

According to the provisions of the F.M.S. Gazette Notification No. 2661 of 27 March 1931, before any proprietary preparation can be admitted to the official list of fungicides approved for the treatment of this disease it must be submitted to the Rubber Research Institute for test.

The conditions of acceptance by the Rubber Research Institute of a material submitted for test are:

a that the proprietors declare the composition of the material (in confidence if so desired), the declaration to be in a form acceptable to the Head of the Pathological Division;

b that the proprietors or their agents agree to deposit a sum to cover the estimated cost of the test. This at present amounts to approximately 400 dollars.

The procedure followed by the Rubber Research Institute for testing is as follows:

a On acceptance, the material is first subjected to an *in vitro* fungicidal test against the pathogen of mouldy rot (*Ceratostomella fimbriata*). This is followed by a phytocidal test. If a material successfully passes these two tests it is then subjected to a field test.

b The proprietors, or their agents, are informed of the location of field test plots.

c A report is provided on completion of the tests, and any balance of the deposit is refunded. On receipt of the guarantee that the composition of the product will remain unchanged from the sample the name of the preparation which has successfully passed the tests is gazetted as an addition to the official list of fungicides approved for the treatment of mouldy rot.

An approved fungicide will be recommended for removal from the official list if complaints as to its inefficacy are substantiated on a retest.

The Rubber Research Institute also reserves the right to publish the results of any test.

TESTING METHODS

The testing methods at present used differ somewhat from those described by Beeley (1936). Whereas prewar the fungicides were all tar acids, tests in recent years have had to be applicable to synthetic materials. Hutchison (1953) describes the promise shown by some of these, especially cationic detergents.

In Vitro TESTS

The method employed consists of seeding standard 9 cm petri dishes containing malt extract media with a sterile water suspension of the spores of *Ceratostomella fimbriata* produced from culture. Sufficient spores to inoculate 50 to 60 plates can be obtained from one tube of culture. After twentyfour hours' incubation at room temperature, the plates are sprayed with the fungicide at a range of concentrations. A small de Vilbiss No. 15 hand atomizer is used and each plate held 6 to 8 inches from the nozzle is given five puffs of the bulb which delivers approximately a total of 1 ml of the fungicide. Each concentration is sprayed on to duplicate plates. Efficacy is judged by comparison first, with unsprayed control plates and secondly, with one of the approved fungicides. After six to seven days further incubation the minimum concentration at which growth is completely inhibited is determined. This test is considered of greater practical significance than the method of seeding plates after they have been sprayed.

While not giving a precise guide to field concentration and performance, *in vitro* tests do rank materials in order of agreement with field ratings. However, from experience in the testing of tar acid fungicides it is known that effective field concentrations are approximately two to three times effective *in vitro* concentrations.

The method of application of fungicides to the seeded plates is open to criticism, depending as it does on the expected delivery of 1 ml with five puffs of the atomizer. The quantity actually delivered may be more or less but the extent of error is not large. An effort to reduce errors which may arise through this has been made by the use of a spray tower which will deliver precise quantities down to 0.2 ml. In the tower the seeded plates are automatically uncovered as they are introduced and as they are removed are recovered. Contamination is very much less than in the hand spraying method but it requires further investigation, and the tower some further modification, before generally accepting the technique.

The following TABLE compares the results of hand spraying with tower spraying.

TABLE I: COMPARISON BETWEEN INHIBITION IN GROWTH OF *C. fimbriata* ON PLATES SPRAYED BY HAND AND IN THE TOWER

Fungicides	*Hand sprayed*		*Tower sprayed*	
Concentration %	0.01	0.005	0.01	0.005
Quantity applied (ml)	1.0	1.0	1.0	1.0
A	– – –	$\frac{1}{8}$ – –	$\frac{1}{8}$ $\frac{1}{8}$ $\frac{1}{4}$	+ + +
B	– – –	$\frac{1}{8}$ $\frac{1}{4}$ $\frac{1}{2}$	$\frac{1}{2}$ $\frac{3}{4}$ $\frac{3}{4}$	+ + +
Control (distilled water)	*Uniform colonies throughout*			

Note *Three plates were sprayed for each treatment*
\+ = *no inhibition*
– = *complete inhibition*
Fractions = *area covered by fungus*

The disparity in results between the two methods in this trial appeared to be due to less fungicide reaching the plates in the tower — a portion of the finely atomised spray being retained on the walls.

PHYTOCIDAL TESTS

Many excellent fungicides are unsuitable for use against mouldy rot because of their toxicity to the newly tapped bark and tests therefore have to be undertaken to guarantee that this is not the case. It must be established that the phytocidal concentration is well above that at which the fungicide is recommended for use. The method of determining this consists of applying the fungicide to newly tapped bark on budded trees, two clones being selected, usually Tjir 1 and PB 86. These two clones represent the most commonly grown and the newly tapped bark of PB 86 is extremely sensitive to damage while Tjir 1 is considerably less so. The dilutions are each applied to four trees, two of each clone, for six tapping days with a day missed between the first and second application in order to discard any obviously damaging the bark.

As the result of these tests the maximum safe concentration is decided upon.

FIELD TESTS

If a material successfully passes *in vitro* and phytocidal tests it is then subjected to field trial. These trials are only undertaken during the wet season when the disease is at its maximum intensity and the site is carefully chosen so that conditions are as favourable to the development of the disease as possible. Ideal sites are not always easy to secure.

Test plots consist of twentyfive trees for each concentration and each tree must have a complete band of the fungus on the tapping cut. The trees are tapped on alternate days and the fungicide applied on the tapping day immediately after collection of the latex. In normal estate practice the fungicide would be applied on the non-tapping day but in the tests the non-tapping day is required for recording the performance of the treatment.

Applications are made by a small hand sprayer, the Vermorel No. 5, delivering spray at the rate of 240 ml per minute in a cone six inches in diameter at a distance of 1 ft from the nozzle. The spray is applied in a single pass round the panel giving sufficient to a 6 inch band to thoroughly wet, but not to the extent of any marked run off. The quantity of spray actually applied will of course depend on the circumference of the tree but an average figure would be 25 ml. Treatment is continued for fourteen applications, or until the disease has been brought under control.

The efficacy of the material is arrived at by recording the state of the disease on each tree on the day following treatment under one of four heads:

Mouldy	=	mould extending along not less than half the length of the tapping cut.
Some mould	=	mould present on less than half the length of the tapping cut.
Trace	=	mould present only as one or two small isolated colonies.
No trace	=	the panel free from all traces of mould.

By alloting numerical values to each of these four categories a figure is obtained which can be used to note the efficacy of a fungicide at any stage of the test. Values that have been chosen for this purpose are:

Mouldy	= 0	Trace	= 2
Some mould	= 1	No trace	= 4

As there are twentyfive trees to each plot a score of 100 on the scale signifies that the disease has been completely eradicated. Perhaps as important as the ultimate control given by a treatment is the rate at which this is achieved, or at least the rate at which the incidence is reduced, and by recording incidence after each treatment in the above manner it is possible to observe this trend. The following graph illustrates the differences in rates of control in one experiment between four fungicides.

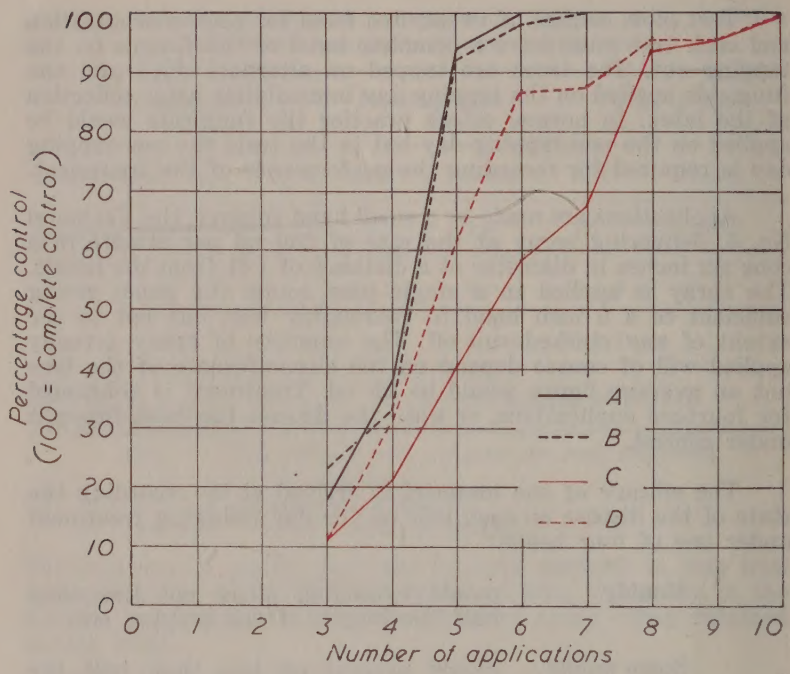

Figure 1. Rate of control of mouldy rot treated with four fungicides.

It is obvious that the most satisfactory material in this trial was fungicide *A* although all have brought the disease under control within the specified fourteen applications.

A material which proves promising after field trial is tested to find whether it has any deleterious effect on properties, such as elasticity or rate of cure, of rubber prepared from latex or scrap of treated trees. It is then given a full scale field trial at the concentrations to be recommended, if possible on more than one site.

Finally, the name of the preparation which has successfully passed all the tests is submitted to the Director of Agriculture with a recommendation that it be gazetted as an addition to the official list of fungicides approved for the treatment of mouldy rot.

SPECIFICATION

In the absence of a specification for tar oil fungicides for mouldy rot treatment the present method of testing materials is laborious, depending as it does, on carrying out field tests.

A first step towards providing the basis for a specification was taken in 1951 when the Board of the Rubber Research Institute financed a programme of work at the University of Bristol Research Station, Long Ashton to assess the fungicidal properties of coal tar distillates. As the result of this work (COLES *et alii* 1954 and 1955) the fractions imparting fungicidal activity have been determined and a method of analysis suggested.

Further experience in the method of analysis and the relation of results in the laboratory to those in the field will be required before a specification can be drawn up. At present therefore, all new materials have to undergo field tests.

Pathological Division
Rubber Research Institute of Malaya
Kuala Lumpur

December 1955

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APPENDIX

Copy of F.M.S. Gazette Notification No. 2661 extracted from Volume XXIII No. 7 of the 27th March, 1931.

No. 2661.—Notice.—

The attention of the Department of Agriculture has been directed to the necessity which exists for the standardisation of disinfectants which are approved for use in the treatment of Mouldy Rot on rubber plantations.

2. From the date of this notice, therefore, in all notices served by the Department of Agriculture under the Plant Disease Prevention Ordinance for the treatment of Mouldy Rot only preparations will be specified which have been subjected to test by an approved authority and proved to be effective.

3. At present the list of such preparation includes:

(1) Agrisol;

(2) Izal — in certain cases.

4. The Department will be prepared to extend the list to include any other preparations which have similarly been proved to be effective, by approved authorities. It should be pointed out that certain other preparations in common use on estates have also been reported to give satisfactory results, but as these have not yet been subjected to systematic tests they are not at present included in the list.

5. The approved authority for carrying out such tests for Malaya will be the Rubber Research Institute and the carrying out of such tests will in future be undertaken by the Rubber Research Institute; application from proprietors of fungicides for the performance of such tests with a view to their inclusion in the list should be addressed to the Director of the Rubber Research Institute accordingly.

6. The tests will be made strictly in accordance with priority of receipt and also in accordance with the capacity of the Institute to deal with them when received.

7. All supplies of disinfectants required for such tests must be furnished without charge by the firms applying for such tests while the actual expenses involved in carrying out the tests must also be borne by the said firms.

8. For further information regarding tests application should be made to the Rubber Research Institute.

9. Proprietors of fungicides and their representatives in Malaya are further notified that preparations which on samples supplied have passed the tests prescribed by the Rubber Research Institute, will only be admitted to the list provided that the proprietors are willing and able to give a guarantee that the formula for the composition of their preparations will remain unchanged.

10. Should any proprietor of an approved fungicide desire to make any change in the composition of the fungicide he must notify the Director of Agriculture and submit samples of the preparation in its new form for further tests if required to do so. Failure to do this may entail the removal of an approved preparation from the list.

Kuala Lumpur
2nd March, 1931.

H. A. TEMPANY,
Director of Agriculture, S.S. & F.M.S.

II THE FUNGICIDAL PROPERTIES OF COAL TAR DISTILLATES:
(i) INVESTIGATION OF THE FUNGICIDAL EFFICIENCIES OF COMPONENTS OF COAL TAR DISTILLATES

By

G.V. COLES and R.J.W. BYRDE

1 The fungicidal efficiencies of fractions of coal tar distillates against Ceratostomella fimbriata, the fungus responsible for the mouldy rot disease of the rubber tree in Malaya, have been investigated. Maximum efficiency has been found to be associated with the high boiling tar acids.

2 Information has been obtained on the extent to which the fungicidal activities of acidic and neutral tar fractions are influenced by boiling range and the method of tar production used.

3 The assessments of fungicidal effect by a laboratory method of bioassay, which is described, have shown correlation with the results obtained in Malayan plantation trials.

Coal tar distillates consist of complex mixtures of acidic (mainly phenolic,) neutral (largely hydrocarbon) and basic (principally nitrogen bases) fractions, boiling over a wide range. The neutral oil and tar acid fractions have already found valuable applications in agriculture and horticulture; neutral tar oil boiling above 230°C forms the basis of the ovicidal winter washes for fruit trees[1], while tar acids (boiling range 250-290°C) have been recommended for potato haulm destruction[2]. Lower boiling tar acids (cresylic acids) are used for soil sterilization, in which fungicidal properties contribute to their effect. In Malaya, Ceylon and elsewhere, tar preparations have been used for many years[3, 4] to control diseases of the rubber tree (*Hevea brasiliensis*), in particular the mouldy rot disease of the newly cut tapping panel caused by the fungus (*Ceratostomella fimbriata* (Ell. and Halst.), Elliott.

Little is known of the efficiency of the various coal tar constituents against plant pathogens. In Malaya, since 1931, proprietary materials have had to pass field tests in order to be approved officially for the treatment of mouldy rot[5]. Large differences in effectiveness have nevertheless been found among approved products[6]. At the request of the Rubber Research Institute of Malaya, work was undertaken to characterize more definitely the fungicidal constituents of tar in order to obtain a chemical basis for the prediction of performance.

EXPERIMENTAL

The fungus *C. fimbriata* was cultured from infected trees in Malaya and sent to Long Ashton.

Three tar preparations designated *A*, *B* and *C*, used in Malaya, were sent for examination to Long Ashton. In plantation tests in Malaya, *A* was the least effective, *B* was intermediate, and *C* was the most effective in controlling the disease. *A* and *B* were single phase oil formulations which emulsified spontaneously on mixing with water, while sample *C* was an emulsion containing about 50 per cent water.

METHOD OF BIOASSAY

The method adopted for laboratory assay was a development of that described by CROWDY[7], standardized as follows:

A sterile peptone-agar medium was prepared with the composition:

Peptone	10 g	Calcium chloride	0.1 g
Dextrose	40 g	Ferric chloride	0.02 g
Potassium dihydrogen phosphate	6.8 g	Agar	30 g
Magnesium sulphate ($7H_2O$)	2.5 g	Water to 1 litre	

The pH of the medium was adjusted to 5.3

The fungicide suitably formulated, was added to a 50 ml portion of the liquid medium at 45-55°C to give the required concentration, and four petri dishes were poured from the mixture and allowed to set. Controls and 'blank' tests for formulating materials, were included. From cultures of the fungus incubated for about ten days on the same peptone agar medium, 4 mm discs of mycelium were cut with a sterile No. 1 cork borer. The dishes containing the materials to be tested were inoculated with the discs of mycelium, and placed in random order in an incubator at 25°C. The mean diameter of each culture was determined by measurement along two previously marked diameters after two days and again after four days of incubation. The effect of each treatment was assessed as the mean percentage inhibition of growth of the fungus over the two to four day period.

COMPARISON BETWEEN LABORATORY AND PLANTATION TESTS

Assessment by this procedure arranged the proprietary materials in the same order as had been found[6] in the plantation trials (TABLE I).

TABLE I: COMPARATIVE PERFORMANCES OF COMMERCIAL COAL TAR FUNGICIDES IN LABORATORY AND PLANTATION TESTS

Fungicide	*Agar method, 2-4 day period*		*Malaya field trial (5% concentration)*	
	Concentration p.p.m.	**Inhibition %*	*Season*	*Efficiency rating*
A	400	65.0	1947	51
	200	39.0	1948	5
	100	24.5	—	—
B	400	65.0	1947	50
	200	33.5	1948	28
	100	27.0	—	—
C	400	97.5	1947	100
	200	54.5	1948	100
	100	30.0	—	—

* *Significant differences:*

$P = 0.05$	11%
$P = 0.01$	14.5%
$P = 0.001$	19%

FRACTIONATION AND TESTS OF FRACTIONS

A preliminary separation of the materials *A*, *B* and *C* into acidic, basic and neutral constituents was made by modifications of the method described for the analysis of tar oil winter washes[8]. Water contents were determined by the Dean and Stark method. The results are shown in TABLE II.

TABLE II: PERCENTAGE COMPOSITION OF COMMERCIAL COAL TAR FUNGICIDES

Material	*Neutral oil*	*Tar acids*	*Tar bases*	*Water*	*Emulsifier, etc.**
A	66.6	9.3	2.1	6.9	15.1
B	64.2	18.2	2.3	5.0	10.3
C	25.5	22.4	1.0	49.8	1.3

* *By difference*

The fractions were insoluble and non-dispersible in water and had to be emulsified before bioassay. After tests with different emulsifiers, a method of emulsification was found that was equally applicable to all the acidic, basic and neutral fractions examined.

To prepare a material for test at 100 p.p.m. in agar, a little more than 0.5 g (x g) of the material was weighed into a 100 ml graduated flask. 20 x ml of a benzene solution containing 7.5 per cent (v/v) of Triton X 155 and 5 per cent (v/v) of Triton B 1956 were then added from a burette, and the flask shaken to dissolve the material. About three volumes of water were added, the flask was shaken vigorously for about a minute to obtain a satisfactory emulsion, and the contents made up to volume. The amount of emulsion required to give 100 p.p.m. of material in the agar was then added to the liquid medium from a sterile 1 ml pipette. For tests at higher levels of dosage, the quantities of materials and emulsifiers taken were adjusted to suitable multiples of x so that the proportion of emulsion present in the agar was always of the same order. The emulsions were prepared 24 hours before use to become 'self sterile'; they mixed readily with the liquid agar and remained stable for some weeks. The emulsifiers caused no inhibition of growth of the fungus at concentrations equivalent to 400 p.p.m. of the test materials.

The results of laboratory bioassay of the fractions separated from the commercial fungicides are shown in TABLE III.

TABLE III: COMPARATIVE PERFORMANCES OF FRACTIONS OF COMMERCIAL FUNGICIDES. PERCENTAGE INHIBITION AT 100 P.P.M. (AGAR METHOD, 2-4 DAY PERIOD)

Fractions	*Fungicide*			*Significant differences* %		
	A	*B*	*C*	*P = 0.05*	*P = 0.01*	*P = 0.001*
Neutral oil	17.0	20.5	31	13.5	18	23.5
Tar acids	65.0	81.0	100	14.0	19	25.0
Tar bases	61.5	52.0	—	27.0	36	46.5
Emulsifiers etc.	0	2.0	0			

In view of the small amounts of tar bases found in the materials examined (see TABLE II), main attention was then focused on the neutral oil and tar acid fractions. Distillation curves were determined by modifications of the Standard Distillation test for Creosote Oils[9] and these are shown in *Figures 1* and *2*. The amount of fractionation occurring in such a distillation is small, with poor separation of the components of a complex mixture. To determine whether future work involving fractional distillation would be justified, however, the distillate of each material was collected in separate portions and these were bioassayed. The results are shown in *Figures 3* and *4*.

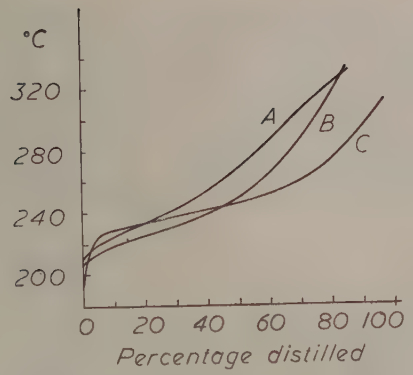

Figure 1. Distillation curves of neutral tar oils from commercial coal tar fungicides A, B and C.

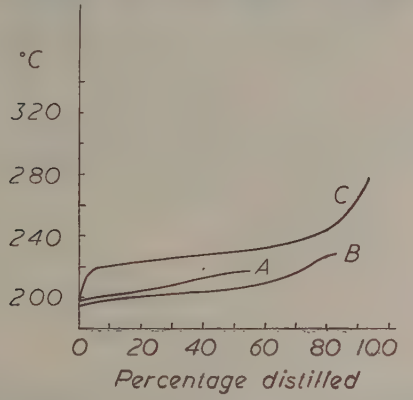

Figure 2. Distillation curves of tar acids from commercial coal tar fungicides A, B and C.

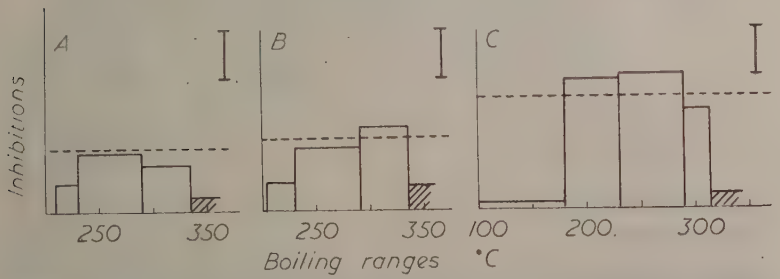

Figure 3. Relative inhibitions of fungus growth by portions from simple distillation of neutral tar oils from commercial fungicides.

----- *Inhibition level of whole materials*

⊓ *Inhibition by fractions*

▨ *Inhibition by residues*

I *least significant difference* (*P* = 0.05)

It appeared from the results shown in TABLE III, and from the plantation results when correlated with analysis that the most fungicidal component of the materials was the tar acid fraction; and from the results shown in *Figures 2* and *4* that the higher boiling tar acids were of most value. To check these conclusions, examination was made of samples of tar acids and of tar distillates containing high proportions of neutral oil, obtained through the courtesy of the Coal Tar Research Association.

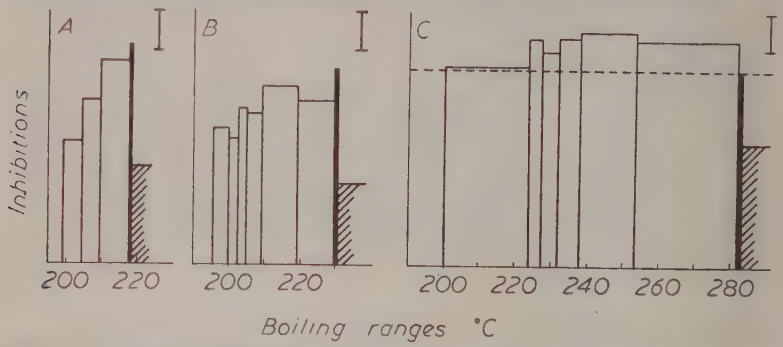

Figure 4. Relative inhibitions of fungus growth by portions from simple distillation of tar acids from commercial fungicides.

---- *Inhibition level of whole materials*
Inhibition by fractions
Inhibition by residues
Inhibition by final distillates
Least significant difference (P = 0.05)

EXAMINATION OF TAR ACIDS AND NEUTRAL OIL DISTILLATES

The materials as received were first bioassayed by the agar method; the results are given in TABLE IV.

TABLE IV: COMPARATIVE PERFORMANCES OF NEUTRAL OIL DISTILLATES AND TAR ACIDS (AGAR METHOD, 2-4 DAY PERIOD)

Material	*Inhibition at 100 p.p.m. %*
Tar oil distillate 1	36.5
Tar oil distillate 2	24.0
Cresylic acids	36.5
High boiling tar acids	53.0

Significant differences:	$P = 0.05$	10%
	$P = 0.01$	13.5%
	$P = 0.001$	17.5%

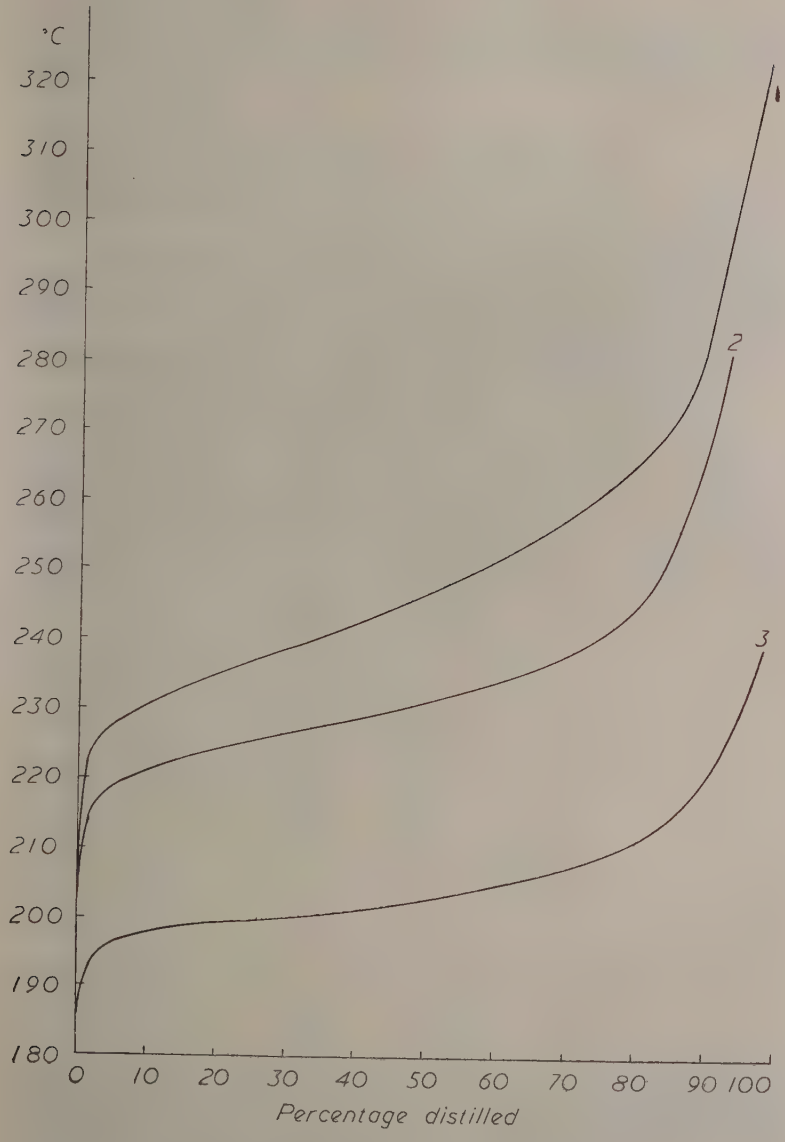

Figure 5. Distillation curves of tar acids: 1 high boiling tar acids. 2 tar acids 'C'. 3 cresylic acids.

Analysis of the tar oils by the method previously used[8] gave the results shown in TABLE V.

TABLE V: PERCENTAGE COMPOSITION OF NEUTRAL OIL DISTILLATES

Material	*Neutral oil*	*Tar acids*	*Tar bases*
Tar oil distillate 1	90.6	3.2	6.2
Tar oil distillate 2	90.2	3.0	6.8

The boiling ranges of the tar acids, determined as before[9], are shown in *Figure 5*, where they are compared with that of commercial sample *C*.

The high boiling tar acids (*D*), the tar oil distillate *1* (*F*) and a blend of equal amounts of each (*E*) were compounded as emulsions containing approximately 50 per cent of water, and

TABLE VI: COMPARATIVE PERFORMANCES OF LABORATORY PREPARED FUNGICIDES IN LABORATORY AND PLANTATION TESTS

Fungicide	*Water content % wt*	*Agar method 2-4 day period*		*Malaya field trial*	
		Concentration p.p.m.	** Inhibition %*	*Concentration %*	*Efficiency rating*
D	53.3	400	98.5	5	98
		200	69.0	2	72
E	53.0	400	93.0	5	94
		200	69.0	2	57
F	50.9	400	77.5	5	36
		200	41.0	2	24
C for comparison	—	—	—	5	98

* *Significant differences:* $P = 0.05$ 11.5%
$P = 0.01$ 15.5%
$P = 0.001$ 20.5%

after laboratory assay were sent to Malaya for plantation trials at the Rubber Research Institute. The method of plantation testing was that used previously[6]. The comparative assessments of the emulsions in field and laboratory are shown in TABLE VI.

The materials were also assessed in Malaya for phytotoxic action (burning of the tree bark). Material *F* was non-toxic at 20 per cent, *D* and *E* were non-toxic at 10 per cent but slightly toxic at 15 per cent.

The results confirm the fungicidal value of the high boiling tar acids and show these to be non-phytotoxic at double the concentration needed for the control of the mouldy rot fungus.

THE EFFECT OF THE METHOD OF PREPARATION OF THE PARENT TAR UPON THE FUNGICIDAL PROPERTIES OF FRACTIONS

It is well known that the chemical nature of coal tar varies considerably with the method of carbonization used. In general, high temperature and long residence-time in the retorts lead to more aromatic tars than lower temperatures and shorter residence-times. Thus the products become increasingly aromatic through the series low temperature (*e.g.* 'Coalite' process) tars, vertical retort and horizontal retort tars, to coke oven tar.

To determine the effect of this on fungicidal efficiency, samples of neutral tar oils of various boiling ranges from the 'Coalite' process were obtained from Messrs Coalite and Chemical Products Ltd. Well fractionated cuts of neutral tar oils distilled in high efficiency columns from typical tars from vertical and horizontal retorts and from coke ovens were supplied by the Coal Tar Research Association. These materials were bioassayed; the results of typical tests are shown in *Figure 6* (*a-d*).

The results of bioassays with typical whole-tar tar acids and whole-tar bases are shown in TABLE VII.

TABLE VII: COMPARATIVE EFFICIENCIES OF FRACTIONS OF SOME TYPICAL COAL TARS

Material	*Tar type*		
	Percentage inhibition at 100 p.p.m. 2-4 day period		
	Low temperature	*Vertical*	*Coke oven*
Tar acids	23.5	31.5	28.5
Tar bases	—	28.5	37.0

Some work has been carried out on the effect of distillation range (without regard to tar source) of the tar acids. In *Figure 7* (*a*) are shown the results of assays on a short series of well fractionated higher boiling tar acids distilled in a high efficiency column and supplied by the Coal Tar Research Association.

Figure 7 (b) shows the results of assays of a longer series of tar acid fractions distilled in less efficient equipment. Differences in efficiency between comparable fractions were much greater in the cuts from the more efficient fractionation.

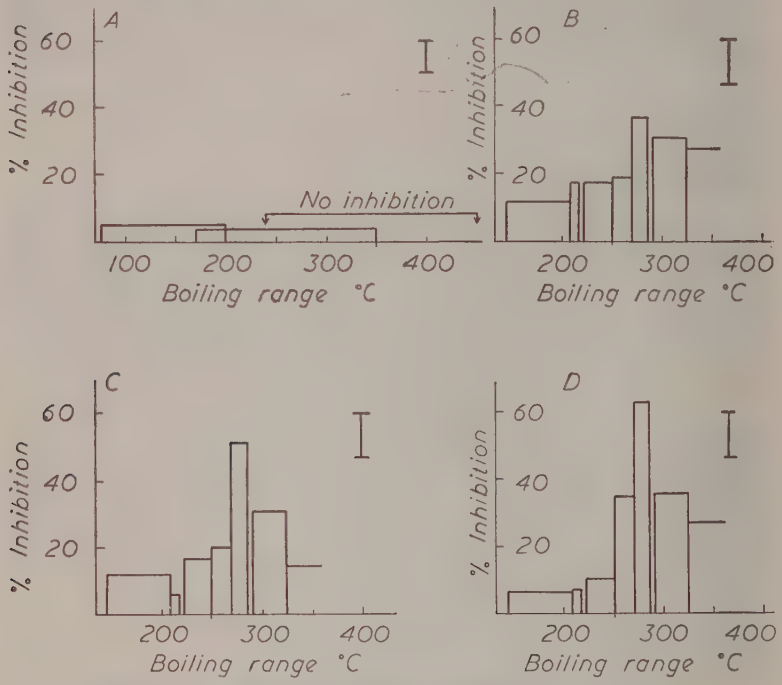

Figure 6. Relative inhibitions of fungus growth by distillation fractions of different neutral oils.
A low temperature tar. B vertical retort tar.
C horizontal retort tar. D coke oven tar.
Inhibition by fraction
least significant difference (P = 0.05)

DISCUSSION

The agar plate method has previously been used in the examination of non-volatile fungicides where a linear relationship between growth and time may be obtained. Mathematical analysis of such results is possible. In the case of tar preparations, which are appreciably volatile, the growth time relationship is no longer linear. Even close cut distillation fractions are extremely complex, and the components of such fractions may be of different fungicidal value and different volatility. Because of these factors, calculated allowances for volatility effects are impracticable. Nor is it possible to apply mathematical analysis to the results

obtained with a range of concentrations of any given fraction. Consequently, the method of assessment of these materials has been based, as already mentioned, on the determination of the

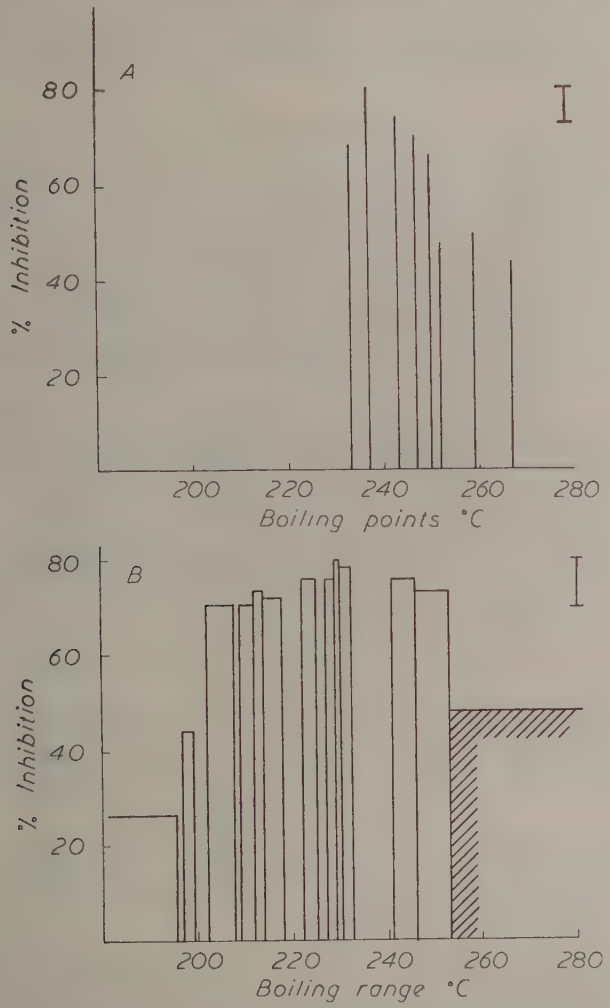

Figure 7. Relative inhibitions of fungus growth by distillation fractions tar acids.
A. distillation fractions from high efficiency column.
B. laboratory distillation

Inhibition by fractions
Inhibition by residues
least significant difference (P = 0.05)

degree of inhibition of fungus growth during the period from two to four days after inoculation. This period has been selected because it has been found that the culture inoculum reaches a steady growth rate after two days, and because the following two days is a similar interval to that in plantation applications, which are commonly made on alternate days, the tree being tapped on the intermediate day.

The results in plantation trials given by the laboratory prepared emulsions (TABLE VI) appear to confirm the earlier indication (TABLE I) that the laboratory bioassay may be relied upon to reflect the field performance of materials. Further comparisons, however, are needed. The results given in TABLE VI also support the contention that the higher boiling tar acids, at least in the materials so far examined, contribute most to field efficiency. The commercial product *C* clearly owes its superiority over products *A* and *B* to the fact that, in spite of its higher water content, it contains a higher proportion of tar acids of greater effectiveness than those in *A* or *B*.

The results of the work on distillation show interesting trends. It is clear that significant differences are detectable, in the laboratory bioassay, between portions of efficiently fractionated materials over quite short ranges of boiling points. (See *Figures 6b, c* and *d* and *Figure 7a*). It also seems that these differences are less significant in portions taken from simple distillations. (See *Figures 3, 4* and *7b*). Further work is required on the effects on field performance of the method of carbonization and of close fractionation, particularly of the tar acids. Little is known as yet about the factors governing phytotoxicity.

The results obtained with well fractionated neutral tar oils (*Figure 6*) indicate that in a narrow boiling range — principally the acenaphthene fraction — there are significant increases in fungicidal value as the carbonization methods become more severe and the oils become more aromatic.

Our experience in the chemical separation of the tar distillates into acidic, basic and neutral fractions, has shown that the methods now used might well be improved and work on alternative methods is in progress.

The results reported have been obtained using one species of fungus. Before the picture of the fungicidal action of tar components is clear it will be necessary to extend the investigations to other plant pathogens. The extent to which the fungicidal activities of tar phenols may be correlated with their germicidal and nematicidal properties is also of interest.

This work has been carried out with funds provided by the Board of the Rubber Research Institute of Malaya, and this paper is published with the agreement of the Director of the Institute.

We are indebted to Dr D. McNeil and his colleagues of the Coal Tar Research Association for valuable discussion and for materials, to Messrs Coalite and Chemical Products Ltd for materials, and to the Rubber Research Institute of Malaya for helpful collaboration.

Thanks are also due to Mrs P.M. Fowles and Miss P.M. Jackson for laboratory assistance, and to Dr J.T. Martin for his interest and support throughout the work.

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(ii) DEVELOPMENT OF A METHOD FOR COAL TAR FUNGICIDE ANALYSIS AND A COMPARISON BETWEEN THE CHEMICAL ANALYSIS OF PROPRIETARY MATERIALS AND THEIR BIOLOGICAL PERFORMANCE IN THE FIELD

By

G.V. COLES, J.T. MARTIN and R.J.W. BYRDE

1 A method of analysis of tar oil fungicides is described. Phenols, bases and neutral oils are determined after the separation of water and formulating solids.

2 The fungicidal efficiencies against Ceratostomella fimbriata of proprietary tar oil fungicides and of constituent fractions have been assessed by a laboratory method of bioassay.

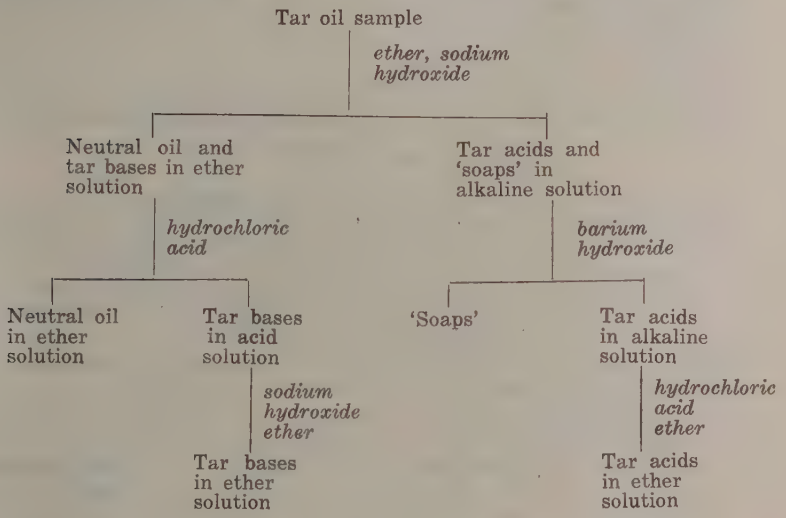
3 The results of bioassay have been correlated with those of chemical analysis. The predominating effect of the phenols in determining the efficiency of the fungicides has been confirmed. No evidence has been detected of a synergistic effect between the phenols and the neutral oil.

The first report[1] described investigations of the fungicidal efficiencies of components of coal tar distillates against *Ceratostomella fimbriata* (Ell. and Halst.), Elliot, the fungus responsible for the mouldy rot infection of the newly tapped bark of the rubber tree. Information was given in the report on the extent to which the fungicidal activities of acidic and neutral tar oil fractions were influenced by boiling range and by the types of tar from which they had been prepared. Maximum efficiency against the fungus was associated with the high boiling tar acids (220°C and above), and it seemed probable that the effect obtained was largely independent of their source. The fungicidal activities of the neutral tar oils, on the other hand, varied according to the method of production of the parent tars, the potencies of the high boiling neutral oils increasing as the carbonization processes became more severe. The tar bases also showed fungicidal activity.

The biological tests were made with tar fractions of known origin and characteristics obtained through the courtesy of the Coal Tar Research Association or from tar distillers, and with fractions separated in the laboratory from commercially available tar oil fungicides. The experience gained in resolving the proprietary materials into their component parts has led to the development of an alternative method for the analysis of tar oil fungicides which is described briefly in this report. The method has been given in more detail elsewhere[2]. An examination is also made of the extent to which the results of the chemical analysis of proprietary materials may be used to assess their biological performance in the field.

(*a*) METHOD OF WINTER WASH ANALYSIS

The fractionation of the proprietary fungicides was first carried out using modifications of the standard method for the analysis of tar oil winter washes[3]. The procedure used in the method is as follows:


Tar oil sample
ether, sodium hydroxide
Neutral oil and tar bases in ether solution
Tar acids and 'soaps' in alkaline solution
hydrochloric acid
barium hydroxide
Neutral oil in ether solution
Tar bases in acid solution
'Soaps'
Tar acids in alkaline solution
sodium hydroxide ether
hydrochloric acid ether
Tar bases in ether solution
Tar acids in ether solution

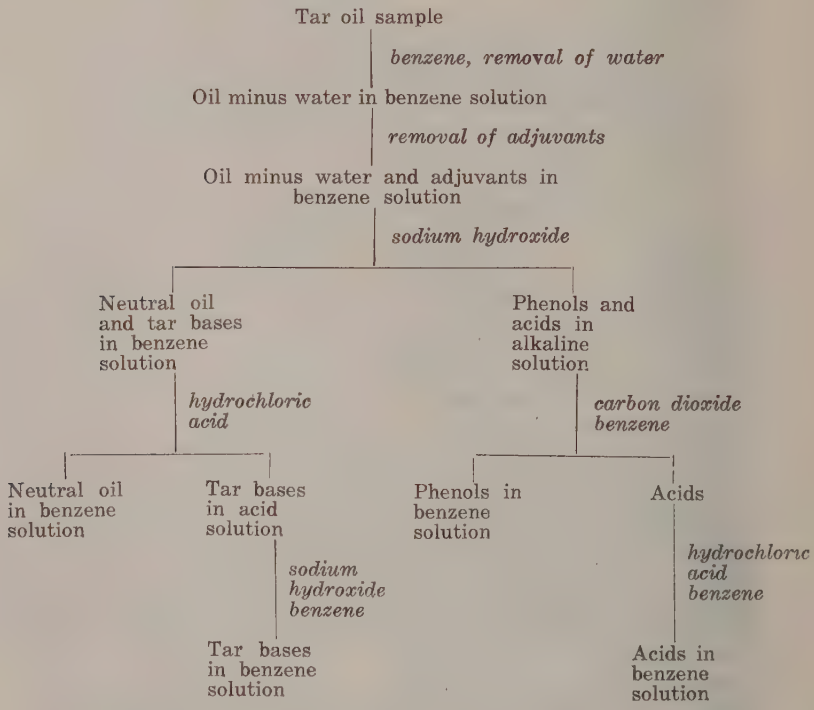
Ether is used as the solvent for the oil, and the tar acids and tar bases are successively removed by extraction with sodium hydroxide solution and aqueous hydrochloric acid. 'Soaps' are separated from the alkaline solution of the tar acids by treatment with solid barium hydroxide. The neutral oil, tar acids and tar bases are recovered in ether solutions which are dried with anhydrous sodium sulphate, the solvent removed and the components weighed. No provision is made in the method for the determination of water content.

When used for the fractionation and analysis of the tar oil fungicides the method was found to have serious disadvantages. Large quantities of ether were required, the drying of the ether solutions was not entirely satisfactory and the solvent was held tenaciously by the tar constituents. Considerable amounts of material separated in the precipitation of the 'soaps' and several additions of barium hydroxide were necessary. The filtration of the separated solids was troublesome, and even after much washing they appeared to retain phenols. At many stages emulsions were formed and at times only partial separations were achieved. The difficulties found with the method seemed to arise

chiefly from the use of ether as solvent and from omitting to remove adjuvants before proceeding to the separation of the main tar constituents.

(*b*) ALTERNATIVE METHOD OF ANALYSIS

The new procedure removes the water and formulating materials at an early stage in the analysis and also differentiates between phenols and other acids originating in the tar or derived from adjuvants. The steps used in the method are as follows:


Tar oil sample
benzene, removal of water
Oil minus water in benzene solution
removal of adjuvants
Oil minus water and adjuvants in benzene solution
sodium hydroxide
Neutral oil and tar bases in benzene solution
Phenols and acids in alkaline solution
hydrochloric acid
carbon dioxide benzene
Neutral oil in benzene solution
Tar bases in acid solution
Phenols in benzene solution
Acids
sodium hydroxide benzene
hydrochloric acid benzene
Tar bases in benzene solution
Acids in benzene solution

Tests with various solvents showed chloroform and benzene to be the most suitable; there was, however, evidence of interaction between chloroform and the alkaline solution of phenols and benzene has therefore been preferred.

The amount of material taken for analysis is adjusted to give approximately 25 g calculated on a water free basis. The water is removed by distillation with benzene using the Dean and Stark apparatus; excessive frothing may be overcome by inserting a condenser, through the jacket of which steam is passed, between the flask and the receiver. During this process adjuvants such as emulsifiers and emulsion stabilizers are precipitated in the oil solution, and these are then removed.

Considerable difficulty was met in effecting the separation of the oil and solids, but this was achieved satisfactorily by continuous extraction of the oil from the flask containing the mixture, using a new extraction assembly designed for the purpose[2]. The flask containing the oil and solids is attached to a liquid extractor tube by a ground glass cone inserted in the tube just below its side arm. A glass disc with holes, fused to the lower edge of the cone, supports a filter pad of cotton wool. The extended stem of a filter funnel passes through the central hole of the disc. The oil in the mixture is washed over into a second flask by condensed benzene falling into the funnel and the extraction is continued until the benzene filtering through the pad is completely colourless. After the recovery of the oil, the solids remaining in the flask or held in the filter may be freed from benzene and weighed.

The separations of acids and bases from the oil solution are then carried out by counter current extractions, in separating funnels, using benzene and 5 per cent (by weight) solutions of sodium hydroxide and hydrochloric acid. Eight alkaline and five acid extractions are made. Phenols are separated from the alkaline solution by passing carbon dioxide before recovering the other acids. In all extractions using the separating funnel, agitation by inverting the funnel fifty times has been preferred to shaking. Water is conveniently removed from the benzene solutions of the phenols, other acids and bases by the Dean and Stark technique. The tar components are obtained by distillation from a water bath of most of the benzene, the remainder of which is eliminated by azeotropic distillation with methyl alcohol followed by drying in an oven at 100°C for a short period.

TABLE I: ANALYSIS OF TAR OIL FUNGICIDES (PER CENT BY WEIGHT) BY THE OLD AND NEW PROCEDURES

Material	*Water*	*Solids*	*Neutral oil*	*Total acids*	*Phenols*	*Other acids*	*Bases*
Standard method for winter washes							
A	*	*	66.6	9.3	*	*	2.1
B	*	*	64.2	18.2	*	*	2.3
C	*	*	25.5	22.4	*	*	1.0
New procedure							
A	6.6	17.2	57.0	—	11.0	1.4	2.3
B	6.7	12.5	55.3	—	18.3	0.8	2.1
C	49.5	2.4	14.9	—	28.9	1.1	0.8

* *Not determined in the method*

Three commercial fungicides (designed *A*, *B* and *C*) sent from Malaya and analysed earlier by the standard method for tar oil winter washes were re-examined by the new procedure. The results are given in TABLE I.

The outstanding differences lie in the values obtained for the neutral tar oils, which are appreciably lower when determined by the new method.

Seven more proprietary fungicides, also sent from Malaya, were analysed by the new procedure. The results are given in TABLE II.

The products differ markedly in their contents of neutral oil and phenols and contain low proportions of bases.

TABLE II: ANALYSIS OF TAR OIL FUNGICIDES (PER CENT BY WEIGHT) BY THE NEW PROCEDURE

Material	*Water*	*Solids*	*Neutral oil*	*Phenols*	*Other acids*	*Bases*
G	46.5	6.2	10.9	34.3	1.6	2.0
H	7.6	7.6	38.6	31.3	1.4	1.3
I	50.0	2.4	19.4	28.0	2.0	0.7
J	54.5	3.7	14.7	23.7	0.5	0.3
K	49.9	5.9	21.9	22.9	0.9	2.1
L	56.2	3.1	6.7	19.4	0.3	0.7
M	4.8	21.7	67.8	8.2	1.0	4.8

BIOLOGICAL TESTS

(*a*) FUNGUS INHIBITION BY THE COMPONENTS OF PROPRIETARY FUNGICIDES

The laboratory method of bio-assay has already been described on page 459. An account of the statistical arrangement and treatment of the tests has been given by Clarke[4].

Materials *A* and *C* and the fractions separated from them by the new procedure were tested in the laboratory against *C. fimbriata*; the results are given in TABLE III.

As already report (page 461, TABLE I) material *C* is appreciably more active against the fungus than material *A*, the inhibition levels, when related to the concentrations of the formulated products, being of a comparable order with those obtained earlier. The most important fungicidal constituents of both *A* and *C* are the phenols; the only other groups to shown an appreciable effect are the bases separated from *A* and the

TABLE III: PERCENTAGE INHIBITION (2-4 DAY PERIOD) OF MYCELIAL GROWTH OF *C. fimbriata* BY FRACTIONS OF PROPRIETARY COAL TAR FUNGICIDES

	p.p.m.	*Material A tested 24/8/54*	*Material C tested 29/6/54*
Original (water free basis)	200	23.5	74.5
	100	19.0	50.5
	50	8.5	28.0
Neutral oil	200	30.0	36.5
	100	17.0	18.5
	50	0.0	10.0
Phenols	200	100.0	73.0
	100	44.5	49.5
	50	10.5	31.0
Other acids	200	30.0	81.5
	100	13.0	49.5
	50	0.0	29.5
Bases	200	61.5	22.5
	100	36.0	18.5
	50	23.5	5.5
*Solids**	200	7.0	0.0
Significant differences:	$P = 0.05$	12.5	8.5
	$P = 0.01$	16.5	11.0
	$P = 0.001$	22.0	14.5

* *Solids were not tested in the statistically designed experiment, but the plates were included in the incubator at the time. Significant differences quoted, therefore, do not apply.*

'other acids' isolated from *C*. The neutral oils show a low activity.

(*b*) TESTS FOR SYNERGISM

Experiments were carried out to determine whether there was any synergistic effect between the constituents occurring in the proprietary products in the greatest proportions, namely the neutral oil and phenols. The fractions isolated (in June 1954) from material *C* by the new method were tested (in March 1955) alone and as mixtures. The results are given in TABLE IV.

The results at both levels of concentration show a steady falling off in inhibition from the values obtained with the more toxic phenols to those given by the neutral oil, and give no indication of a synergistic effect. A second experiment, using the tar acids *D* and neutral oil *F* sent to Malaya for field test (page 466), also failed to show an interaction between these components.

TABLE IV: PERCENTAGE INHIBITION (2-4 DAY PERIOD) OF MYCELIAL GROWTH OF *C. fimbriata* BY PHENOLS AND NEUTRAL OIL ALONE AND IN ADMIXTURE

	Phenols %	*Neutral oil* %	*Mean growth* mm	*Inhibition* %
At 104 p.p.m.	100	0	2.63	74.7
	80	20	4.00	61.4
	60	40	4.25	59.1
	40	60	4.88	53.0
	20	80	6.38	38.5
	0	100	6.38*	38.5
At 55 p.p.m.	100	0	5.00	51.8
	80	20	5.63	45.8
	60	40	6.50	37.3
	40	60	6.75	34.9
	20	80	7.00	32.5
	0	100	7.13	31.3
Control	—	—	10.38	—
Significant differences:		$P = 0.05$	1.46	14.1
		$P = 0.01$	1.95	18.8
		$P = 0.001$	2.58	24.9

* *One plate lost.*

(*c*) FUNGUS INHIBITION BY PROPRIETARY MATERIALS

The proprietary fungicides *G*-*M* whose analyses are given in TABLE II were also tested against the fungus, both in their original miscible oil or emulsion forms and also on a water free basis. *p*-Phenyl phenol, at 25 p.p.m., was included in some of the tests as a standard fungicide. The results are given in TABLE V.

Judged from the tests on the formulated products at 400 and 200 p.p.m., the materials show a decline in toxicity from *G* to *M*. Further examination of the results show that the products may be separated into two groups, one consisting of *G*, *H*, *I* and *J* and the other *K*, *L* and *M*. Comparable concentrations of products within each group do not differ significantly from each other in toxicity (at $P = 0.05$), while those of the first group are significantly more active than those of the second (at $P = 0.05$).

TABLE V: PERCENTAGE INHIBITION (2-4 DAY PERIOD) OF MYCELIAL GROWTH OF *C. fimbriata* BY PROPRIETARY COAL TAR FUNGICIDES

Material	*As formulated*			*Water free basis*		
	p.p.m.	*Tested 4/1/54*	*Tested 2/2/54*	*p.p.m.*	*Tested 29/3/54*	*Tested 10/4/54*
G	400	100	—	200	84.0	—
	200	65.5	56.5	100	60.5	—
	100	—	34.5	50	39.5	—
H	400	—	91.0	—	—	—
	200	52.5	52.0	—	—	—
	100	14.5	—	—	—	—
I	400	85.5	—	200	—	81.5
	200	54.5	62.5	100	—	60.5
	100	—	37.5	50	—	34.0
J	400	85.5	—	200	74.5	—
	200	51.0	53.5	100	57.0	—
	100	—	33.0	50	44.5	—
K	400	60.0	—	200	66.5	—
	200	43.5	28.5	100	57.0	—
	100	—	24.0	50	38.0	—
L	400	69.0	—	200	—	69.0
	200	42.0	42.0	100	—	55.0
	100	—	15.0	50	—	24.0
M	400	52.5	—	200	—	26.5
	200	31.0	45.0	100	—	17.0
	100	—	28.5	50	—	4.0
p.-Phenyl phenol	25	38.0	45.0	—	—	—
Significant differences: $P = 0.05$		11.5	7.5		9.0	7.0
$P = 0.01$		13.5	10.0		12.0	9.5
$P = 0.001$		20.0	13.0		15.5	12.5

An analysis of the results was made to determine to what extent the fungicidal activities of the products could be ascribed to particular constituents determined by the new method. At the level of 400 p.p.m. the partial correlation coefficient of the percentage inhibition with the percentage phenols content (eliminating the variation within the range of materials in the percentage of neutral oil) was 0.866, significant at $P = 0.01$. At the same level of concentration, the partial correlation coefficient of the inhibition due to the neutral oil, eliminating the effect

of the phenols, was 0.086, certainly not significant. At the 200 p.p.m. level, the corresponding coefficients were 0.811 and 0.127, the former being significant at $P = 0.05$ (and almost significant at $P = 0.01$). At 100 p.p.m. neither coefficient was significantly different from zero.

DISCUSSION

The alternative method of analysis proposed represents, in our view, a step forward in the chemical examination of tar oil fungicides. Not only does it give a measure of the contents of water and formulating solids, but the early removal of these ensures a more satisfactory separation of the acids, bases and neutral oil. Difficulty, however, was still met in the analysis of some of the proprietary products. The tests on *G*, *I*, *J* and *K* went smoothly and total recoveries within the range of 95.5 to 102.7 per cent were obtained; with *H* and *L* solids continued to be thrown out after the initial separation procedure, probably due to the incomplete removal of water. The high recovery value (108 per cent) obtained with *M* was attributed to the retention of solvent by the tar components. The method, however, is promising and with further experience may provide the basis of a standard test for materials of this kind.

The phenols clearly play a predominant part in determining the efficiency of the proprietary products. Both the determination of phenols and the laboratory bioassay have placed the fungicides in an order of efficiency which has followed closely the order observed from tests with earlier consignments on the control of the disease in the field. The phenols contents, however, although of value in assessing the potential effect, do not give more than an approximate measure of toxicity since the particular phenols used may influence the level of control obtained. The products *I* and *J* for example, differ in their phenols contents but show the same order of inhibition in the laboratory tests, probably due to the use of the more toxic phenols in the formulation of *J*. The results from this and earlier work suggest that a minimum content in the formulated product of about 25 per cent of the more active phenols (boiling point 220°C and above) is necessary to achieve a reasonable degree of efficiency under the normal conditions of application in the field.

The possibility of a synergistic effect between the tar components has been considered but no evidence of an interaction between the chief constituents, the phenols and neutral oil, has been detected. The tar bases show some activity, but occur in insufficient quantities in the fungicides to contribute materially to their overall toxicities. The bases may, however, influence the activity of the other components, but this has not been examined.

A feature of the laboratory bioassay has been the reproducibility of the levels of inhibition of the fungus obtained in

comparable tests over an appreciable period. *p*-Phenyl phenol was included in some of the experiments as a standard fungicide; more recently we have preferred to use *o*-phenyl phenol which at concentrations of 75, 15 and 3 p.p.m. has consistently given percentage inhibitions of the order 80, 45 and 20 respectively. Work in progress on the fungicidal activities of individual phenolic, neutral and basic tar components will be reported later.

The work has been carried out with funds provided by the Board of the Rubber Research Institute of Malaya and is published with the agreement of the Director of the Institute. We are indebted to members of the Institute for their cooperation.

We also express our thanks to Mr G.M. Clarke for the statistical interpretation of the data and to Miss P.M. Jackson and Miss N.M. Waugh for valuable laboratory assistance.

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THE ANALYSIS OF COAL TAR FUNCICIDES*

By G. V. COLES†

An account is given of the difficulties encountered in applying an existing standard method of analysis of coal tar preparations to the separation of the constituents of certain fungicides used for treatment of rubber-tree disease. The method which was developed to overcome these difficulties is described. The scheme of analysis involves the preliminary removal of water and formulating materials, using simple apparatus, and is followed by countercurrent extractions. Recovery of the separated groups of constituents appears to be good and will enable an assessment to be made of their contribution to the biological activity of the original materials.

Introduction

An investigation has been made recently of the fungicidal value of certain coal tar preparations used in dressing the tapping panel of the rubber tree.[1] The contributions made to this property by the various groups of constituents in the preparations had to be determined. It was therefore necessary to separate these groups in quantities sufficient, first, for biological testing and then, so far as possible, for isolation of those individual constituents which were of most fungicidal value. Because it was hoped that it might be possible to develop a chemical method of evaluation of the fungicides, it seemed desirable to employ a procedure which could form a basis for a future standard method of analysis.

A method of analysis[2] already in use for coal tar preparations employed as fruit-tree winter washes was therefore used for preliminary work. This separation involves the use of ether as solvent for neutral constituents, tar acids are removed by washing with aqueous sodium hydroxide and tar bases are removed by similar treatment with aqueous hydrochloric acid. The concentration of the sodium hydroxide solution used is based to some extent on findings regarding the effect of the emulsifying agents used in some types of preparations.[3] The specified method for breaking emulsions formed during the extractions is by the addition of saturated sodium chloride solution. Any soaps present are regarded as being removed in the alkaline solution of tar acids, from which they are precipitated by the addition of barium hydroxide, after which the tar acids are liberated by the acidification of the solution, and removed by ether extraction. The ethereal solutions of all three groups of constituents are dried with anhydrous sodium sulphate, the ether removed by distillation and the recovered separated materials weighed. The overall recovery which should be obtained is not specified.

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† Present address: P.O. Box 227, Kampala, Uganda.

Experimental

A. Experience of the standard method of analysis

When applied to a commercial 'Neutral Tar Oil' supplied for winter wash preparation, the foregoing method presented little difficulty, giving a total recovery of about 97%.

Three commercial tar-based fungicides, which had been found in practice to possess widely differing degrees of fungicidal efficiency, were next analysed. Two of these were of the 'miscible oil' type (emulsifying on the addition of water), and the third was an emulsion. The method could only be applied with difficulty. The following were the major difficulties encountered:

(*a*) At all stages of the extractions, emulsions were obtained, some of which had to be set aside for several days before even partial separation took place. Very large quantities of solvent and reagents were required for all the extractions; in consequence, unwieldy volumes of liquid had to be handled a number of times during the procedure.

(*b*) Considerable amounts of material separated when the baryta additions were made as specified for soap preciptation, several additions of baryta were necessary before separation appeared complete, and in some cases the ensuing filtrations were troublesome. When the precipitates thus obtained were acidified, in attempting to separate and determine the acids contained in the soap, the materials which eventually separated always appeared to contain phenols; the separated materials were often solids or semi-solids for which no solvents were found to permit their extraction for subsequent determination of the amounts present.

(*c*) Where the aqueous extracts were neutralized, gelatinous materials (which were probably derived from formulating materials used as emulsion stabilizers) separated out, and interfered with the removal of the tar acids and tar bases.

(*d*) The method used for drying the separated constituents seemed unsatisfactory in three respects. Firstly, the bulky ether solutions had to be concentrated somewhat before using a drying agent; these solutions were saturated with water and consequently contained about 1.5% of water; it appeared that this water caused the loss of a proportion of the steam-volatile constituents—particularly the phenols—during this concentration. Secondly, the rapid evaporation of the solvent during filtration of the solution from the drying agent chilled the filters to such an extent that the resulting visible condensation of atmospheric moisture was sufficient to reintroduce enough moisture to separate as droplets when the ether was removed. Thirdly, the drying agent always retained sufficient of the various constituents to be coloured by them and smell strongly of them even after prolonged washing with ether.

(*e*) Ether appeared to be retained very tenaciously by the main tar components.

These difficulties seemed to be due in the main to the presence of the formulating materials and to the use of ether as a solvent. Other objections to the method were:

(1) The recoveries obtained were considered unsatisfactory.

(2) The scheme of extraction employed was not a true countercurrent one and therefore was unlikely to give very thorough separation of the mixtures.

(3) No differentiation is made between true phenols and other acidic material either originating in the coal tar or introduced in the formulating materials.

(4) Tar oils are not completely stable chemically. It is well known that some of the acidic constituents tend to be readily oxidized by air in presence of alkali. The neutral constituents are known to contain free radicals[4] which are believed to be responsible for some of the resins which are found to separate on exposure to light and air. It therefore seemed very necessary to reduce the time taken for the separations.

B. Development of an alternative scheme of separation

In evolving a more suitable method of separation and analysis, a comparison was first made of the purely solvent properties of four potentially suitable volatile solvents for coal tar constituents. Samples of two high-boiling neutral oil fractions, and of one high-boiling tar acid fraction were mixed with ether, light petroleum, b.p. 40-60°, benzene and chloroform, and set aside for 48 hours. Light petroleum, as had been expected, was found to precipitate appreciable quantities of all the materials and was clearly unsuitable. The other three solvents all left very small residues; chloroform was the best, but not markedly so, No improvement in separation of phases was obtained, however, by using benzene or chloroform in the extractions by the original method.

The key to an improved method was discovered when an attempt was made to improve the overall recovery by supplying the most obvious omission from the old method—the determination of the water content of the materials. The Dean & Stark method was used, with toluene as the entrainment solvent, and gave satisfactory results; in addition, it was noted that material insoluble in toluene was precipitated in each case—sometimes in considerable quantity. This removal of water has proved a very convenient means of separating formulating materials, and of proceeding to a satisfactory scheme of extraction. The formulating additives in commercial tar preparations usually consist of surface-active agents and emulsion stabilizers. When the preparations contain appreciable amounts of tar acids, the former are restricted to soaps and synthetic anionic surface-active materials; however made or added these are insoluble in water-free solutions of tar oils in certain organic solvents. Stabilizers, such as gelatin and casein, are also precipitated from mixtures with these solvents on the removal of water.

With this observation, the choice of solvent became limited to benzene and chloroform, because each could be used as a solvent for the azeotropic removal of water by the Dean & Stark technique, while ether was too miscible with water to be employed; both solvents precipitated the surface-active and emulsion-stabilizing materials when all water had been removed. Further, by continuing to use these solvents in all subsequent extractions during the separations, it now became possible to dry the separated constituents of the tar oils in their solutions, before removal of the solvent, by the same technique and without any undesirable introduction of drying agents.. Later in the work, it was found necessary to reject chloroform as a solvent because it reacted with the sodium hydroxide solutions of phenols. After the precipitated formulating materials had been removed, benzene proved to be a very suitable solvent during all the subsequent extraction processes. Preliminary work with benzene solutions of high-boiling tar-oil constituents showed that this solvent could be removed from the tar constituents on the water-bath, to give satisfactory recoveries of these constituents, by making use of the fact that benzene forms an azeotrope with methanol. (The standardized procedure developed from this work is included below in an appendix.)

A further problem which had to be overcome at this stage was the removal of the formulating materials precipitated by the removal of water from the mixtures. In most cases it proved impossible to filter these from the benzene solution; they varied from bulky gelatinous masses through casein precipitates, dyes, brittle resins and gummy semi-solids to small adherent deposits. Eventually a new type of extractor was devised to handle this miscellany of substances. The assembly is shown in Fig. 1. A long-stemmed funnel (A) is a sliding fit in the central hole of a perforated glass disc fused on to the bottom of a B-34 cone (B), and around it a cotton wool filter-pad is packed. A flask (C) of water-free mixture of benzene, tar constituents and precipitated formulating material is connected to this cone, and the joint is reinforced with springs. The funnel is pushed down to touch the bottom of the flask. The other cone (D) is connected to a flask (E) of dry benzene for extraction, and a condenser with a silica-gel guard-tube to exclude moisture is fitted to the socket. The benzene in the lower flask is boiled on a water-bath, so as to extract all benzene-soluble matter from the specimen of water-free preparation. The extraction is continued until the benzene overflowing to the boiler is quite colourless. It has been found convenient to carry out this extraction under reduced pressure by connecting the assembly to a suitable vacuum system through the guard-tube: the rate of extraction is increased several times thereby, but should not be so rapid as to disturb the precipitate unduly, lest the filter become clogged.

FIG. 1.—*Extractor for removal of soluble from precipitated material*

This extractor has been found very convenient and it may perhaps be found useful for other similar problems. For quantitative work when the soil is in suitable form, a sintered glass disc could be sealed to funnel and cone and used in place of the removable pad.

The next stage was the improvement of the extraction of tar acids and tar bases from solutions presumed to contain solely those portions of the fungicides originating in coal tar.

In the absence of formulating materials which interfered with extraction, it immediately became practicable to use sodium hydroxide solution (5%), for the removal of tar acids, and to use a countercurrent scheme of extraction. This scheme is shown in Fig. 2. Each extraction was accomplished by 50 successive inversions of the separating funnels, which have been reported[5] to be adequate for the equilibration of two phases. This technique created none of the difficulties from emulsion-formation which were so objectionable in the original method. The combined aqueous extracts finally obtained were then treated with CO_2 until all the phenols were released, which were then extracted with benzene. The solution of liberated phenols in benzene was obtained in two days from the commencement of alkali extraction. The aqueous solution left after extraction of the phenols was then neutralized with hydrochloric acid and any other acids so liberated were also extracted with benzene.

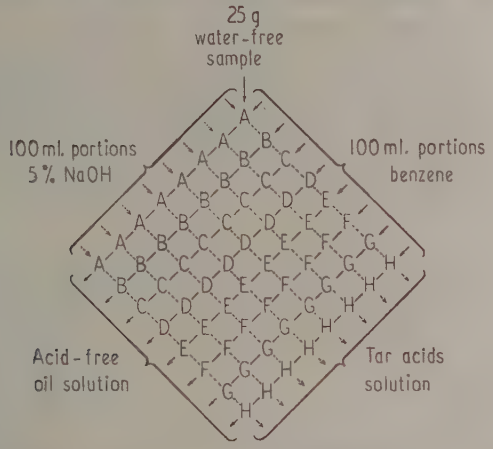

FIG. 2.—*Extraction scheme used for analysis of tar oil fungicides*

The benzene solution free from tar-acid constituents was reconcentrated, and a similar scheme of countercurrent extraction by 10% aqueous hydrochloric acid was employed to remove tar bases. The combined acid extracts were neutralized with sodium hydroxide and the tar bases were extracted with benzene.

The benzene solutions of phenols, other acids, tar bases and neutral tar constituents were all dried by the Dean & Stark method, the benzene then being removed by distillation.

Results

The new procedure developed in the manner outlined above proved so superior to the old method that few complete comparative analyses of typical commercial formulations were made by both methods. The results of two of these are shown in Table I. Results of analyses of a number of proprietary materials by the new procedure are reported elsewhere.[6]

Table I

Comparative analyses (% weight) of tar fungicides by standard and new techniques

Fungicide type	Method	Water	Formulating solids	Neutral tar oil	Phenols	Other organic acids	Tar bases	Recovery %
Miscible oil	Standard	—	—	66.6	9.3	—	2.1	78.0
	New	6.6	17.2	57.0	11.0	1.4	2.3	95.5
Emulsion	Standard	—	—	25.5	22.4	—	1.0	48.9
	New	49.5	2.4	14.9	28.9	1.1	0.8	97.6

Discussion

The recovery obtained by the new method appears to be far more satisfactory than that by the standard procedure and the method does not present any great difficulty. At no stage in the procedure is the material heated above 100°, and it is considered that chemical changes in the separated materials should not be excessive, so that they remain suitable for any subsequent biological tests. Should it be used as the basis of a standard method of analysis, it would be desirable that collaborative work should be carried out by several laboratories on mixtures of known composition to determine the differences likely to be found between different operators, especially in the stages involving solvent removal.

Results of biological tests associated with the work now reported have been published elsewhere.[6] It should be mentioned here, however, that these results appear to suggest that the scheme of separation now described has the merit of providing separated constituents whose fungicidal activities, considered in conjunction with the extent to which they are found in the original material, provide a reasonably satisfactory correlation with the fungicidal activity of the original. While of no particular merit to the analyst, this may make the method of some value to workers on allied problems.

Appendix

Procedure

All reagents and solvents should be of analytical quality. Benzene should be dried by the Dean & Stark tenchnique, and benzene and methanol should be redistilled through a Widmer column. Precautions should be taken throughout the method to avoid the inhalation of benzene vapour.

Weigh sufficient sample to provide about 25 g. of water-free material into a short-necked flask with B-34 joint. Add sufficient benzene to act as entrainment solvent. Connect a Dean & Stark trap of suitable size, and a suitable condenser. (Use a trap with a tap for emulsion-type preparations.) If the sample is

found to froth during the removal of water, fit a steam-jacketed froth breaker between the flask and the trap: a short Liebig condenser with the jacket connected to a steam generator has been found suitable. Boil the solvent on the water-bath until at least 1 hour has passed without further water being recovered. Calculate the water content of the sample. Draw off and retain the water from emulsion-type formulations.

Prepare an extractor (as shown in Fig. 1) with a cotton wool pad, and weigh the assembly. The cotton wool used should be previously extracted with benzene and methanol and dried to constant weight at 100°. Connect the flask of water-free material (C) to the cone containing the pad, and connect a flask (E) of benzene to the other cone. Complete the assembly as shown in Fig. I. Boil the flask of benzene on a water-bath, using reduced pressure if necessary, until the benzene layer in the flask (C) of emulsifying materials is colourless. Set aside the flask (E) of extracted benzene-soluble matter. Remove the long-stemmed funnel from the extractor, dry and weigh any solids adhering thereto. Pipette off sufficient of the benzene in the filter cone and the neck of the original flask (C), running this liquid into a larger B-34 neck flask (F), and transfer flask C to the other cone of the extractor, connecting flask F to the filter cone. Remove as much benzene as possible from flask C on the water-bath, collecting it in flask F together with any material thereby washed from the filter pad. Connect flask F to a solvent-recovery system and remove as much benzene as possible on the water-bath. Reconnect flask F to the filter cone; add sufficient methanol to flask C to remove all remaining benzene as azeotrope and remove all possible solvent on the water-bath, allowing it to fall through the pad into flask F. Drive off all this solvent from flask F through the solvent recovery system. Place both flasks in an oven at 100° with air circulation for 15 minutes; dry the extractor and cotton wool pad to constant weight at 100°. Weigh all the extracted formulating materials so far separated.

The flask E of extracted material should now be reconnected to an extractor and fresh filter pad, and treated in the same manner as C. Removal of emulsifying materials is regarded as satisfactory when less than 1% of the solids removed by the first extraction remains after any subsequent extraction.

Concentrate the benzene solution, if necessary, to about 120 ml. after solids have been satisfactorily removed and transfer to a 300-ml. separating funnel. Reserve the recovered benzene for use as extraction solvent in the scheme of extraction illustrated in Fig. 2, which is now begun on the concentrated benzene solution. The water removed from emulsion type preparations and previously set aside is added to the aqueous layer in the first extraction. Each separating funnel throughout the scheme

is not shaken, but inverted 50 successive times. After sufficient extraction stages (eight appear adequate) the benzene extracts and the aqueous layers are each combined. The benzene solution is concentrated again to about 120 ml. and the recovered benzene used as the extraction solvent in the subsequent scheme of extraction of bases, which resembles that shown in Fig. 2, although five stages appear sufficient and 10% aqueous hydrochloric acid is employed.

Bubble carbon dioxide through the combined sodium hydroxide washings in a large separating funnel until all phenols appear to be liberated. Extract exhaustively with 100-ml. portions of benzene, and pass more CO_2 until all phenols are removed. Now neutralize the aqueous solution with hydrochloric acid and extract with benzene any organic acids liberated. Combine the aqueous hydrochloric acid solutions of tar bases and neutralize with sodium hydroxide before extracting with benzene.

Dry the benzene solutions of tar phenols, other tar acids, tar bases and neutral constituents by the Dean & Stark method. Concentrate each and transfer the concentrate to a tared 150-ml. flask; remove benzene by distilling from a water-bath through a solvent-removal column until solvent comes off at less than 1 drop per minute. Weigh roughly, and add methanol sufficient to remove all the benzene remaining as azeotrope. (Assume the whole contents of the flask to be benzene; the azeotrope contains 61.4 mol.-% of methanol.) Mix well and reconnect to a solvent-removal column. Remove solvents until the rate of removal is less than 1 drop per minute. (If necessary, aliquot portions of the benzene solutions should have been taken so that no more than 10 g. of any constituent will remain after solvent removal.) Transfer the flask while still hot to an oven at 100°, fitted with air-circulation, for 15 minutes. Cool for 30 minutes and weigh.

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The University of Bristol
Department of Agriculture and Horticulture
Long Ashton Research Station
Bristol

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